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Dendritic Spines: The Stuff That Memories Are Made Of?

Two new studies explore structural changes of nerve cells as a potential mechanism for memory formation by studying synaptic reorganization associated with motor learning.

Sonja B. Hofer[1] and Tobias Bonhoeffer[2]

One of the most impressive abilities of the mammalian brain is its capacity to constantly learn new skills, integrate new experiences and form long-lasting memories. How the brain accomplishes this, in view of the vast amount of information we are faced with every day, has been the subject of intensive study for many decades. Two new studies [1,2], together with work published earlier last year [3], now provide strong evidence in support of a structural basis for information storage in cortical circuits.

It is generally believed that changes in the synaptic connections between neurons play a major role in learning and memory formation. While short-term memory might rely mainly on the strengthening and weakening of pre-existing synapses [4,5], long-term storage of information is thought to require structural reorganization of neuronal networks, the formation of new synapses and the loss of existing connections [6]. The first evidence for the importance of functional and structural synaptic plasticity for learning and memory came from seminal studies in the sea slug *Aplysia*. These showed that simple learning processes, like habituation or sensitization, are based on the weakening or strengthening of synaptic connections [7]. Importantly, long-term effects were accompanied by structural changes such as an increase in the number of synapses [6].

Might similar mechanisms of structural reorganization of synapses also account for more complex forms of learning and memory formation in the mammalian brain? Because of recent advances in genetic labelling techniques and imaging methods, it is now possible to repeatedly image fluorescently labelled structures such as axonal boutons and dendritic spines — the pre- and postsynaptic components of excitatory synapses — in the intact cortex of living animals, either through a chronically implanted cranial window or by repeated thinning of the skull [8,9]. The dynamics of synapses thus can be followed while the animal experiences altered sensory input or learns a behavioural task, and structural reorganization can be correlated with changes in neuronal activity and behaviour. Studies using this method demonstrated that a subset of synapses remains highly dynamic even in adult cortex, and boutons and spines appear and disappear continuously [9–14].

These ongoing structural changes have been hypothesized to endow cortical networks with the capacity to translate novel experiences into anatomical traces. And indeed several studies have recently confirmed that altered sensory experience induced by whisker removal, eye closure or retinal lesions causes synaptic and even axonal reorganization in the cortex of rodents and primates throughout life [3,8,15–18]. The two new studies [1,2] have now pursued this idea further by studying spine dynamics during different forms of learning and experience, providing further evidence for structural changes as a possible basis for long-term memory.

Yang *et al.* [1] looked at the effect of two different plasticity paradigms — a novel somatosensory experience and a form of motor learning — on the generation, maintenance and elimination of dendritic spines in mouse cortex. The animals were either presented with an enriched environment that provided new somatosensory input or they learned to maintain their posture on a rotating rod (rotarod) that was continuously accelerated. The structural changes were remarkably similar for both paradigms: after two days new spines were generated in somatosensory or motor cortex, respectively, of which a small percentage persisted over the next weeks. The percentage of new spines that remained stable increased with longer training or exposure periods. Some animals that had learned the motor task were reassessed three months after the initial training. They still mastered the skill of running on the rotarod better than naïve mice. Interestingly, while there was no additional spine growth when repeating the original protocol, a slightly different training paradigm, running backwards on the accelerating rod, resulted in the formation of new spines. In other words, new learning induced new spines, but recall of previously learned skills did not.

The other study, by Xu *et al.* [2], reinforces the idea that motor learning causes lasting structural changes

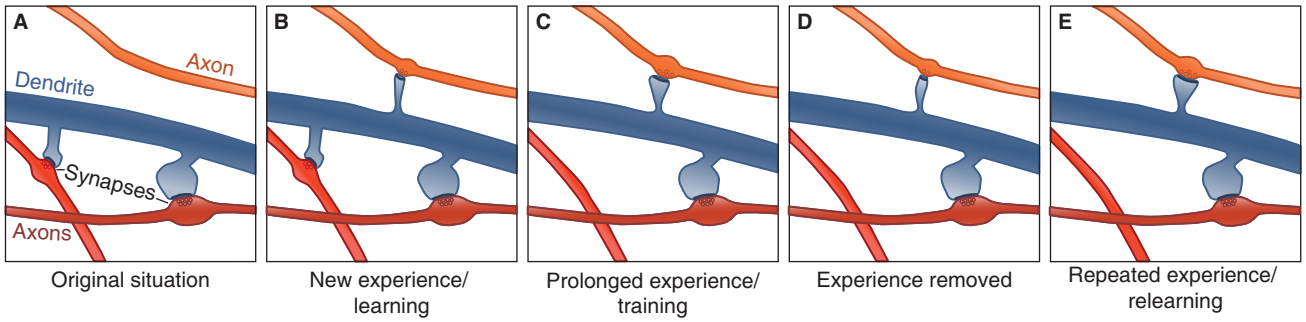

Figure 1. A model for synaptic reorganization associated with learning and memory formation.

(A) A dendrite forming synapses (consisting of presynaptic dendritic spines and postsynaptic axonal boutons) with several axons. (B) During learning of a task or a novel experience, new synapses are formed through the growth of new spines (pointing upwards in the figure) [1–3,15]. (C) Ongoing training or exposure leads to stabilization of new synapses and the concurrent enlargement of the respective spines [1–3,15] as well as the elimination of some pre-existing connections (left downward-pointing spine in the figure) [1,2]. (D) After cessation of experience or training, some of the new spines remain stable over long time-periods [1–3], while their synapses may become weakened or inactivated (indicated by a smaller and thinner spine) [3]. (E) When repeating the same task or experience, no new spines are grown [1–3], but weakened synapses (thinner spines) might become re-activated quickly (spines get thicker), leading to faster (re-)learning [3].

on the level of dendritic spines. In an elegantly designed 'single seed reaching task', mice had to learn to retrieve and handle a food grain with one paw. The authors showed that this was paralleled by a surprisingly rapid generation of new spines in the motor cortex contralateral to the trained paw. Increased spine formation was observed as early as one hour after the first training session, and the percentage of new spines correlated with the mice's performance. A two-week training in the seed reaching task proved very effective in stabilizing the learning-induced spines, of which about 40% could still be found four months later, providing a potential basis for the equally persisting motor skills. In line with the experiments described above [1], exposing the pre-trained animals to the same reaching task did not result in additional structural changes, while spine reorganization was increased when a new food-handling task was learned.

Both groups [1,2] found in addition that only longer training caused increased spine elimination. An attractive explanation is that the removal of inappropriate connections provides the basis for behavioral refinement observed in mice that had more practice.

The main results of these two studies [1,2] are strikingly similar and at the same time extend earlier work from our laboratory using monocular deprivation as a cortical plasticity paradigm in mouse visual cortex [3]. Our study found that new spines that were induced by altered sensory input were preserved and outlasted the transient experience. Importantly, cortical adaptation to a second, similar experience occurred faster [19] and was not accompanied by further structural changes. This strongly suggested that the persistent spines may provide the basis for information storage, and may thus account for the phenomenon that early experience facilitates later (re-)learning [20].

The two new studies [1,2] corroborate this idea, and advance it by correlating structural changes with behavioural output. The authors state that their work investigates true learning whereas earlier approaches examined non-physiological manipulations. Here, we would disagree and argue that learning also comprises situations in which the animal adapts to non-physiological conditions such as altered sensory input or pairing of a tone with an electric shock. But regardless of how exactly one defines learning, the notable similarity of functional and structural changes accompanying both, adaptation to sensory manipulation and motor learning, argues that both are useful models for studying the mechanisms underlying learning and memory in general.

The three studies [1–3] together make a very strong case for the role of lasting synaptic reorganizations in the formation of durable memories. Specific sets of new synapses arise during new experiences or the acquisition of new skills, and might provide the foundation for their retention (Figure 1). The last years have shown remarkable progress in determining structural changes that occur with synaptic plasticity paradigms. However, to date all work on structural imaging *in vivo* was restricted to showing correlations between structural changes and plasticity, and only in the most superficial part of the cortex. What remains to be clarified is if the new synapses directly change the neurons' processing of task-related information rather than merely providing modulatory input. To prove that new spines and synapses encode long-term memories and are essential for their retention, we need to show what information they carry, e.g. by *in vivo* calcium imaging of learning-induced spines, and whether memory is affected by their loss. The ongoing improvements of methodology in the field raises hope that these challenging experiments may be feasible in the not too distant future.

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Behavioral Neurobiology: How Larval Fish Orient towards the Light

Orientation of animals towards or away from light is a simple behavior commonly found in the animal kingdom. A recent study using zebrafish larvae has revealed the underlying neural logic of this primal choice behavior, by differential use of the retinal ON- and OFF-pathways.

Kaspar P. Mueller and Stephan C.F. Neuhauss

Motile organisms exploring their environment are constantly confronted with a plethora of stimuli that demand the animal to take appropriate decisions. Such navigational decisions are apparent in animals with simple nervous systems and also in neonates, hence at least some decision rules need to be independent of experience and hardwired. For visual animals, the single most important stimulus is a light gradient, which requires the animal to decide whether to veer towards or away from the source of light. This process is called positive or negative phototaxis, depending on its direction. Phototaxis is a widespread phenomenon that is even observed in a rudimental form in prokaryotes. In eukaryotes, phototaxis is thought to have evolved independently at least eight times [1].

In this issue of *Current Biology*, Burgess *et al.* [2] report a study that beautifully dissects the rules leading to the observed behavior of phototaxis in zebrafish larvae. Zebrafish larvae are well suited for such research, as phototactic behavior in this species is expressed early in development, independent of learning and attention, with the added bonus that the zebrafish is a genetically tractable model system with deficient mutant strains available. Zebrafish larvae navigate towards or away from a target light spot using two simple motor patterns — routine turns and slow swims (scoots). During positive phototaxis, larvae first turn towards and thereupon rapidly approach the source of light by an increased frequency of scoot movements compared to baseline activity. In contrast, under negative phototactic conditions, larvae first turn away from the light source and then slowly veer away, but do not exhibit increased scoot rates. The decision to react to a light gradient by positive or negative phototaxis depends on relative target intensity — that is, it depends on both the intensity of the target *and* the pre-adapting light. By dissecting the phototactic response in its constituent parts and revealing the triggering stimuli for each of these, Burgess *et al.* [2] found an explanation for this counterintuitive finding.

A promising place to look for a neurobiological basis of phototaxis is the retina. In all vertebrate retinas visual information is channeled into an ON-pathway and an OFF-pathway: the first is activated by an increase of light intensity, while the second is activated by a decrease or dimming of light [3,4]. In order to test the involvement of these separate retinal pathways, Burgess *et al.* [2] made clever use of a mutant line that is selectively disrupted in the ON-pathway. Behavioral analysis of this *no optokinetic response c* (*nrc*) mutant line [5,6] allowed them to separately study the contribution of these two retinal pathways to the phototactic response. In contrast to their wild-type siblings, *nrc* mutants did not show any elevation of scoot movements during positive phototaxis, showing that approaching the target light is mediated by the ON-pathway, and that 'light ahead' is the crucial signal to activate the approach mechanism (Figure 1A). This also explains why scoot frequency is not elevated during negative phototaxis, as there is no increase in illumination and therefore no ON-signal is present while the larva swims away from the light source. Pharmacological treatment with either a serotonin reuptake inhibitor or a serotonin receptor antagonist further showed that serotonin signaling is a key part of this neuronal pathway, likely in linking sensory input to motor output.